Influence of Certain Amino Acids upon the Enzymic Hydrolysis of Starch

DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

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This investigation is a continuation of the study of amylases begun by Professor H. C. Sherman in 1907. The author is greatly indebted to Professor Sherman for many helpful suggestions received from him during the course of this work.

INFLUENCE OF CERTAIN AMINO ACIDS UPON THE ENZYMIC HYDROLYSIS OF STARCH

In 1893 Effront[1] stated that asparagine accelerates the hydrolysis of starch by malt and taka-diastase. A few years later[2] he obtained similar activation by addition of certain proteins and of a boiled cold water extract of barley. In 1904 he reported[3] that asparagine aspartic acid, hippuric acid, creatin, creatinin and the peptones increased the action of malt extract, while succinamide, the amines and their salts, and acid amides generally act unfavorably. This he found for several starches of different origin. He also found, however, that the more favorable the conditions for the production of an optimum amount of sugar, the less marked is the effect of the amino acids.

Ford[4], working with malt, found asparagine to be without effect on the activity of the enzyme. The apparent activation by amino acids and acid salts obtained by other investigators, he ascribes to the neutralization by these compounds of alkaline impurities in the starch.

According to Terroine and Weill[5] saccharification by pancreatic juice is greatly accelerated by alanine, glycine, leucine, valine, histidine, arginine, tyrosine, phenylalanine, aspartic acid and glutamic acid. The activating power which they found the digestion products of protein to possess is, they think, most probably due to the amino acids formed.

More recently Rockwood[6] has investigated the effects of nitrogeneous substances on the hydrolysis of corn starch by saliva and concludes that glycine, tyrosine, anthranilic acid and its *meta* and *para* isomers, aspartic acid, hippuric acid, proteins (serum albumin and gelatin) and amines of the methane series increase saccharification, whereas the amides (acetamide, propionamide and urea), sulphanilic acid, asparagine, succinamide and succinimide show no such

[1] Mon. sci., *41*, 266 (1893).
[2] Compt. rendu., *120*, 1281 (1895).
[3] Bull. soc. chim., [3] *31*, 1230 (1904); Mon. sci., [4] *18*, 561 (1904); Compt. rendu. soc. biol., *57*, 234 (1904).
[4] J. Soc. Chem. Ind., *23*, 414 (1904).
[5] Compt. rendu. soc. biol., *72*, 542 (1912).
[6] Jour. Amer. Chem. Soc. *39*, 2745 (1917).

effect. He also found that glycine and aspartic acid activate pancreatic extract.

Because of the lack of agreement among previous workers as to whether amino acids do or do not activate amylases, and in view of the fact that past investigations have seldom covered more than one enzyme, a systematic study of the influence of amino acids upon the action of several amylases, in purified as well as in their natural or commercial forms, seemed desirable. An attempt has been made in the present work to throw some light on the manner in which the amino acids act.

EXPERIMENTAL

Apparatus and Materials.—The glassware was of the best quality, carefully selected and when not in use was kept filled with water. Just before using, it was washed with soap, rinsed ten times with tap water, three times with ordinary and once with triple distilled water. A Freas thermostat bath with a variation of $\pm$ 0.005° was used for all digestions at 40°C. Digestions at other temperatures were carried out in a small bath kept constant within $\pm$ 0.15° by means of an Ostwald regulator.

Merck's "soluble" starch according to Lintner purified by washing 9 times with ordinary distilled and 6 times with triple distilled water has in all cases been used as substrate. The salts employed as activators were C. P. recrystallyzed several times. The amylase preparations and other enzyme containing materials tested were (1) pancreatic amylase preparations Nos. 58, 59, 60, 77B, 81B and 11B, (2) commercial pancreatin No. 8, (3) malt amylase preparation No. 155, (4) malt extract, (5) aspergillus amylase preparations Nos. 22, 22b, and 23, (6) commercial takadiastase, and (7) fresh saliva. Triple distilled water was used for making starch dispersions of activators, enzymes, etc., and for rinsing all glassware.

Method.—The method[7] of testing the influence of the amino acids is briefly as follows. An amount of air-dry starch equivalent to the required amount of anhydrous material is weighed out, mixed with a little cold water, dispersed by pouring into boiling water (about 80 c.c. per gm. of starch) and boiled for about 3 minutes. This is

[7] Sherman, Kendall and Clark, Jour. Amer. Chem. Soc., *32*, 1082 (1910).

transferred to 100 cc. cylinders, neutralized with 0.01 N sodium hydroxide solution and the salts[8] most favorable for the action of the amylase added. The dispersions are then made up to 100 c.c. so that the concentration of starch is exactly 1 per cent, mixed thoroughly by stirring and placed in the 40° bath to reach the desired temperature. In the meantime, the enzyme solution is prepared and the required amount pipetted into dry flasks. The starch dispersions are then poured into the flasks containing the enzyme at intervals of 15 seconds and the flasks placed in the 40° bath. At the end of 30 minutes, enzymic action is stopped by pouring 50 c.c. of Fehling solution into the digestion mixtures, at intervals of 15 seconds and in the same order in which the starch was poured on the enzyme. The amount of reducing sugar formed is determined by immersing the flasks in a boiling water bath for 15 minutes. The cuprous oxide is filtered into weighed Gooch crucibles, washed with hot water, alcohol and ether, dried at 100°, and weighed. Glycine and alanine being quite soluble were dissolved in a small volume of water and added to the starch paste, after it was poured into the cylinders and before being made up to volume. Since tyrosine and phenylalanine are difficultly soluble, the amount of each used was added to the water in which the starch was dispersed and boiled with it. To show whether this variation in procedure affected the action of the amino acid on the enzyme, digestions were carried out in which equal amounts of asparagine were added before boiling in some cases and after cooling in others. Activation due to asparagine was the same in both cases. The same test was made with aspartic acid with the same result. In these experiments the amino acids were made neutral to rosolic acid with 0.01 N sodium hydroxide solution.

MEASUREMENT OF THE INFLUENCE OF DIFFERENT AMINO ACIDS

Tables I-IV show the influence of carefully neutralized glycine, alanine, tyrosine and phenylalanine, added separately and in combination with a second amino acid, upon the rate of hydrolysis of "soluble starch" by different enzymes. The reducing sugar formed

[8] Sherman, Thomas and Baldwin, *ibid.*, *41*, 231 (1919). Pending further investigation the substrate is prepared in the same manner for the action of saliva as for pancreatic amylase.

by enzymic hydrolysis is chiefly maltose, but since small amounts of glucose may also be present the results are stated in terms of the weight of cuprous oxide resulting from the reduction of Fehling solution by the sugar or sugars present. The amounts of enzyme used in the experiments were so regulated as to result in the transformation of about ⅕ of the starch into sugar.

TABLE I

Effect of Glycine and Glycine Plus Aspartic Acid on the Enzymic Hydrolysis of Lintner Soluble Starch

Amino Acid, Mg.		Cuprous Oxide, Mg.						
Glycine	Aspartic Acid	Purified Pancreatic Amylase (No. 59)	Commercial Pancreatin (No. 8)	Saliva	Purified Malt Amylase (No. 155)	Malt Extract	Aspergillus Amylase (No. 23)	Commercial Takadiastase (No. 7)
0	0	246	227	316	260	277	222	292
50	0	280	243	334	270	286	226	292
100	0	284	245	341	273	283	224	292
0	50	279	242	344	269	280	223	289
50	50	279	247	334	268	281	224	292
Activation due to glycine		38	18	25	13	9	4	0

TABLE II

Effect of Alanine and Alanine Plus Glycine on the Enzymic Hydrolysis of Lintner Soluble Starch

Amino Acid, Mg.		Cuprous Oxide, Mg.						
Alanine	Glycine	Purified Pancreatic Amylase (No. 58)	Commercial Pancreatin (No. 8)	Saliva	Purified Malt Amylase (No. 155)	Malt Extract	Aspergillus Amylase (No. 23)	Commercial Takadiastase (No. 7)
0	0	273	281	319	285	248	272	279
50	0	310	293	339	295	251	279	282
100	0	318	301	352	301	257	287	290
0	50	320	300	355	299	249	277	280
50	50	318	296	360	305	256	284	286
25	25	317	298	351	299	250	273	283
Activation due to alanine		42	20	33	16	9	15	11

TABLE III

Effect of Tyrosine and Tyrosine Plus Asparagine on the Enzymic Hydrolysis of Lintner Soluble Starch

Amino Acid, Mg.		Cuprous Oxide, Mg.						
Tyrosine	Asparagine	Purified Pancreatic Amylase (No. 60)	Commercial Pancreatin (No. 8)	Saliva	Purified Malt Amylase (No. 155)	Malt Extract	Aspergillus Amylase (No. 23)	Commercial Takadiastase (No. 7)
0	0	282	294	323	248	252	261	287
50	0	318	317	353	262	266	273	299
0	50	316	312	352	256	255	263	291
25	25	317	317	355	263	259	269	296
50	50	322	319	362	263	263	270	297
100	0	—	—	356	—	—	—	—
0	100	—	—	364	—	—	—	—
Activation due to tyrosine		36	23	30	14	14	12	12

TABLE IV

Effect of Phenylalanine and Phenylalanine Plus Asparagine on the Enzymic Hydrolysis of Lintner Soluble Starch

Amino Acid, Mg.		Cuprous Oxide, Mg.						
Phenylalanine	Glycine	Purified Pancreatic Amylase (No. 58)	Commercial Pancreatin (No. 8)	Saliva	Purified Malt Amylase (No. 155)	Malt Extract	Aspergillus Amylase (No. 23)	Commercial Takadiastase (No. 7)
0	0	267	293	207	244	250	252	288
50	0	300	306	222	251	253	261	290
100	0	303	307	225	256	256	263	293
0	50	309	312	225	253	254	259	291
50	50	309	312	225	257	257	264	293
25	25	308	311	225	260	255	261	292
Activation due to phenylalanine		36	14	18	12	6	11	5

The data given in the above tables show an undoubted increase in the activity of purified pancreatic amylase, pancreatin, saliva, and purified malt amylase in the presence of any one of the four amino acids investigated or of any two of them whose joint effects were tested. The apparent activation is not so marked in case of the less

sensitive enzymes, malt extract, takadiastase and aspergillus amylase. It is also true that the acceleration of hydrolysis by the amino acids is somewhat greater for the purified form of the enzyme than for the natural or commercial material in which the enzyme is accompanied by other constituents of the tissue or secretion in question. It will be observed that, in general, the four amino acids here discussed as well as asparagine and aspartic acid previously studied[9] behave in a similar manner. The above results show no evidence that the addition of two amino acids to the same digestion mixture causes greater activation than would result from a corresponding concentration of one of them. The following combinations have been tested: aspartic acid and asparagine, glycine and aspartic acid, tyrosine and asparagine, phenylalanine and asparagine, alanine and glycine.

Since some investigators have held that the activating effect of amino acids is attributable to their presence inducing a more favorable hydrogen ion concentration in the digestion mixture, we have determined electrometrically the hydrogen ion concentrations of our mixtures with and without neutralized amino acid, with the results shown in Table V. It is evident that the reaction of our mixtures is not changed by the addition of the neutralized amino acids to any significant degree and therefore that the favorable effect of the amino acid upon the enzyme action is due to some other cause or causes.

TABLE V

Hydrogen Ion Concentration in Solutions with and without Neutralized Amino Acids

Amino Acid	Soln. activated as for pancreatic amylase P_H+	Soln. activated as for malt amylase P_H+	Soln. activated as for aspergillus amylase P_H+
None	6.90	4.46	4.91
50 mg. glycine	6.86	4.50	5.01
50 mg. alanine	6.88	4.45	4.91
50 mg. tyrosine	6.86	4.46	4.89
50 mg. phenylalanine	6.88	4.48	4.91

MODE OF ACTION OF THE AMINO ACID

Several possible explanations of the favorable influence of the amino acids may be suggested. (1) There may be a direct accel-

[9] Sherman and Walker, Jour. Amer. Chem. Soc., *41*, 1867 (1919).

erating effect upon the enzyme-starch reaction. (2) The amino acid may combine with one or more products of digestion which, if free, might retard the enzyme action. (3) The amino acid may protect the enzyme against the deleterious influence of some accidental or unknown impurity. (4) The amino acid may retard hydrolytic destruction of the enzyme.

Is the Action Direct?—It is conceivable that the amino acid may directly facilitate the interaction of the enzyme and substrate. Until our knowledge of the mechanism of enzyme action is further developed this suggestion can only be approached by somewhat speculative discussion, or experimentally by a process of elimination of other possibilities.

Does the Effect Depend upon Some Reaction with the Products of Digestion?—Since the activity of an enzyme is often diminished by the accumulation of the products of its action, it might be suggested that the amino acids exert their favorable influence through combining with some product or products of the hydrolysis which might otherwise combine with the enzyme itself thus reducing its activity, or it might, if remaining free in the solution, tend to bring the hydrolysis to equilibrium. To test this point, the effect of the addition of 100 mg. of pure maltose to the starch paste, with and without glycine, on hydrolysis by pancreatic amylase was determined. Similar experiments in which a certain amount of a hydrolytic mixture was substituted for pure maltose were carried out as follows. One gm. of starch was digested for 1 hour at 40°C. by pancreatic amylase preparation, at the end of which time the enzyme was destroyed by boiling. The effect of 25 and 50 c.c. of this digested mixture on hydrolysis with and without glycine was tested. The fact that known amounts of sodium chloride and phosphate were added with the digested material was taken into consideration, and the volumes of these activators added to the substrates containing the hydrolytic mixtures were adjusted accordingly. Correction being made for their reducing power, both pure maltose and the hydrolytic products of the starch were found under the conditions of these experiments to be without measurable effect upon the activity of the enzyme used, showing that the favorable influence of the amino acid cannot be explained in this way.

Does the Amino Acid Protect the Enzyme against Some Accidental or Unknown Deleterious Influence?—Aside from the possibility of correcting an unfavorable hydrogen ion concentration which has already been excluded as an explanation of our results, it is possible that the amino acid may act by protecting the enzyme from some active but unknown deleterious influence. This is illustrated by the following experiments with cupric sulphate.

Protective Action of Amino Acids against Cupric Sulphate.—These experiments were designed to show whether the deleterious effect upon amylase activity of such a heavy metal salt as copper sulphate could be wholly or in part overcome by the presence of an amino acid. In the experiments the results of which are given in Table VI, the cupric sulphate and amino acid were added to the cooled starch paste and thoroughly mixed before pouring onto the enzyme solution.

TABLE VI

Action of Amino Acids in Protecting Purified Pancreatic Amylase from the Deleterious Effect of Copper

Amino Acid	Mg.	Conc. $CuSO_4$ in starch paste	Cuprous oxide Mg.
None		0	274
None		0.00003 M	58
Alanine	100	0.00003 M	298
Asparagine	100	0.00003 M	293
Glycine	100	0.00003 M	304
Glycine	50	0.00003 M	295
Glycine	100	0	308

The above data show that a 0.00003 M concentration of cupric sulphate in the digestion mixture diminished the activity of pancreatic amylase by about 78 per cent. This is in accordance with results recently obtained in this laboratory by Sherman and Wayman.[10] However, upon the addition of 0.1 per cent of amino acid, not only is the inhibiting influence of the cupric sulphate counteracted, but there is an increase in saccharification almost equal to that which occurs in the presence of amino acid and absence of copper. Further experiments were performed in which the cupric sulphate was added directly to the enzyme solution in two concentrations, 0.0035 M and 0.00003 M and the efficiency of 0.1 per cent glycine in the substrate in reactivating the enzyme was studied. Some results are given in Table VII.

[10] Jour. Amer. Chem. Soc., *43*, 2454 (1921).

TABLE VII

Reactivation of Pancreatic Amylase by Glycine after Inactivation by Copper

Glycine Mg.	Conc. $CuSO_4$ in starch paste	Conc. $CuSO_4$ in enzyme solution	Conc. $CuSO_4$ in digestion mixture	Cuprous oxide Exp. 1 Mg.	Exp. 2 Mg.	Exp. 3 Mg.
0	0	0	0	280	252	264
100	0	0	0	316	—	300
100	0	0.0035 M	0.00003 M	98	—	—
0	0	0.0035 M	0.00003 M	4	—	—
0	0	0.00003 M	0.00000018 M	—	209	—
100	0	0.00003 M	0.00000018 M	—	260	184
100	0.00003 M	0.00003 M	0.00003 M	—	260	182

Numerous experiments showed that the inactivation of the enzyme by the copper and its reactivation by amino acid were considerably influenced by time and temperature. In Expt. 2 of Table VII the solution stood for 12 minutes before testing; in Expt. 3 it stood for 55 minutes. The influence of temperature is illustrated in Table VIII.

TABLE VIII

Effect of Temperature on Reactivation of Pancreatic Amylase by Glycine after standing for 20 minutes in 0.00003 M cupric sulphate solution

Glycine Mg.	Temp. of enzyme sol. after 20 min.	Cuprous oxide Mg.
0	23°C	115
100	23	142
0	12	175
100	12	213

As to the bearing of these experiments with cupric sulphate upon the question whether the role of amino acids is that of a direct accelerator of the enzyme action or rather that of a protector which increases the amount of work done by the enzyme through preventing its deterioration, it cannot be doubted that they establish the possibility of a very marked protective effect without precluding the additional possibility of a more direct action upon the enzyme.

Evidently with a low concentration of copper ions in solution these react with amino acid forming copper-amino ions[11] more readily than with the enzyme; and moreover, when the copper ion has already

[11] J. T. Barker, Trans. Faraday Soc., *3*, 188 (1908).

acted upon and inactivated a part of the enzyme, it apparently may still be taken up by the amino acid, and the enzyme thus freed from the copper may become active again.

Does the Amino Acid Act by Retarding the Hydrolytic Destruction of the Enzyme?—Another possibility is that the amino acid may act by preventing or retarding the deterioration of the enzyme in its aqueous solution. The very rapid deterioration of water solutions of pancreatic amylase, particularly when highly purified, and the influence of the sodium chloride and secondary phosphate (regularly used to "activate" this enzyme) in retarding the deterioration have been discussed in previous papers from this laboratory.[12] Since the deterioration of enzymic activity, while greatly retarded, is not entirely prevented by the presence of the salts, it is not improbable that the favorable influence of the amino acid may be due at least in part to a further protection of the enzyme from deterioration in the aqueous dispersion in which it acts. In studying this point three methods of investigation have been used. (1) Enzyme solutions with and without amino acid were allowed to stand for a definite length of time at known temperatures in absence of substrate and the resulting loss of activity compared. (2) The effect upon activation due to amino acids of varying the temperature at which the enzyme was allowed to act was determined. (3) A similar series of experiments was carried out in which the length of time of digestion as well as the temperature was varied.

The method of determining the effect of an amino acid in retarding the deterioration of purified pancreatic amylase in water solution was as follows. 10 mg. of enzyme were dissolved in 100 c.c. of water at ice box temperature containing 5 c.c. M NaCl and 2.5 c.c. M/50 Na_2HPO_4. 10 c.c. were pipetted into each of four 25 c.c. beakers, the second of which contained 20 mg. of alanine. At intervals of eight minutes 20 mg. of alanine were added to the third and fourth beakers. None was added to enzyme solution in fourth beaker. These stood at room temperature for 33 minutes after the last addition of alanine. Then 0.6 c.c. from each beaker was pipetted into flasks and substrate poured on and digestions carried out as usual. The substrates digested by the amylase solutions to which the amino acid had been added contained 1.2 mg. alanine put in with 0.6 c.c.

[12] Sherman and Schlesinger, Jour. Amer. Chem. Soc. Series of papers (1912-1916).

of enzyme, therefore, to equalize conditions, 1.2 mg. were added to starch dispersions acted upon by the control solution. When tested the solutions to which alanine had not been added were as active as those containing 0.2 per cent of the amino acid.

The procedure was then varied, the enzyme being allowed to stand at two different temperatures in solutions containing salt, phosphate and alanine in different combinations. Results are shown in Table IX. In experiments 1 and 2, indicated in following table, the temperature was accurately controlled at 22°C., in experiment 3, at 40°C.

TABLE IX

Effect of 0.1 Per Cent Alanine in Retarding Deterioration of Pancreatic Amylase in Solution at 22°C and 40°C.

Alanine (0.1%)	NaCl (optimum conc.)	Na_2HPO_4 (optimum conc.)	Cuprous Oxide, Mg. Exp. 1 22°C	Exp. 2 22°C	Exp. 3 40°C
absent	present	present	274	—	144
present	present	present	275	—	198
present	absent	absent	133	—	—
absent	absent	absent	98	—	—
absent	present	absent	—	273	—
present	present	absent	—	272	—
absent	absent	present	—	238	—
present	absent	present	—	269	—

The above table indicates that the amino acid does retard the hydrolytic destruction of the amylase. Solutions of pancreatic amylase (containing optimum concentrations of sodium chloride and phosphate) which have stood 1 hour at 40°C. show about ⅓ greater amylolytic activity when alanine has been added to the solution in advance. Under similar conditions except that the solution is kept at 22°C. instead of 40°C. there is no measurable difference, probably because the deterioration at this temperature in the presence of optimum concentrations of salts is small in either case. It is probable that at 22°C. the amino acid has little or no effect on the rate of hydrolysis of starch by amylase, since, as will be shown later, the activation decreases markedly with temperature. It further appears from Table IX that amylase solutions to which the usual NaCl and Na_2HPO_4 have not been added are protected by alanine even at 22°. At this temperature the amino acid is apparently capable of replacing either the sodium chloride or the phosphate without altering the

activity of the solution, but not both salts. In these experiments, however, the conditions were such as to result in greater deterioration than occurs in our ordinary tests of enzyme activity both because of longer exposure of the enzyme to water and because of the absence of its substrate.

If protection against hydrolytic destruction is a partial explanation of the increased activity of enzymes in the presence of amino acids, it would be logical to expect that any condition favoring the hydrolysis of the enzyme molecule, such as a higher temperature or subjection to a given temperature for a longer period, would cause greater apparent activation by the amino acid. To test this point, a series of experiments was planned in which 30 minute and 60 minute digestions by pancreatic amylase with and without amino acid were carried out at temperatures ranging from 30°-75°C. Table X shows the results obtained with glycine at different temperatures when 0.6 c.c. of 0.01 per cent enzyme solution acts upon 100 c.c. of substrate for 30 minutes and when one-half the amount acts upon the same amount of substrate for 60 minutes. The experiments were repeated with phenylalanine replacing glycine, the results of which are given in Table XI. The purified pancreatic amylase preparation employed in the phenylalanine experiments was about a year older than that used with glycine and only two-thirds as active, consequently, one-third more of the solution was added to each digestion mixture.

TABLE X

Effect of Variation of Temperature on Activation of Purified Pancreatic Amylase by Glycine

	30 minute digestions Cuprous Oxide, Mg.				60 minute digestions Cuprous Oxide, Mg.			
Temp.	50 mg. glycine present	No glycine	Activation due to 50 mg. glycine		50 mg. glycine present	No glycine	Activation due to 50 mg. glycine	
30°C	155	136	19	14%	142	114	28	24%
40°	252	220	32	14%	287	248	39	12%
50°	366	311	55	17%	343	259	84	32%
55°	393	291	102	35%	369	214	155	72%
57°	363	251	112	45%	367	167	200	120%
60°	380	183	197	107%	320	84	236	281%
65°	176	58	118	203%	99	24	75	312%
66.5°	110	30	80	266%	52	19	33	173%
70°	55	24	31	129%	18	8	10	125%
75°	0	0	0	0%	0	0	0	0%

TABLE XI

Effect of Variation of Temperature on Activation of Purified Pancreatic Amylase by Phenylalanine

Temp.	30 minute digestions Cuprous Oxide, Mg.				60 minute digestions Cuprous Oxide, Mg.			
	50 mg. phenyl-alanine	No phenyl-alanine	Activation due to 50 mg. phenylalanine		50 mg. phenyl-alanine	No phenyl-alanine	Activation due to 50 mg. phenylalanine	
30°C	183	164	19	12%	173	143	30	21%
40°	291	258	33	12%	290	247	43	17%
50°	340	255	85	33%	300	194	106	55%
55°	291	181	110	60%	226	94	132	140%
60°	134	66	68	103%	105	37	68	184%
65°	42	25	17	68%	22	12	10	83%
70°	10	7	3	43%	6	4	2	50%

The results of the above experiments afford striking evidence that deterioration of the enzyme with increase in temperature is retarded by amino acids. For the 30 minute digestions, beginning with an increase at 30°C. of 19 mg. of cuprous oxide or 14 per cent, the activating effect of glycine reaches a maximum of 197 mg. of cuprous oxide or over 100 per cent at 60°C. Above this temperature the acceleration as represented by increase in mg. of cuprous oxide declines sharply, although the percentage activation continues to increase up to 66.5°C. The rapid falling off in activity after 60°C. is doubtless due to coagulation of the amylase which is not prevented by the amino acid.

The experiments with phenylalanine show the same general effect though not as marked as in the case of glycine. Maximum activation occurs at 55°C. instead of 60°C. after which the decline is rapid. It will be observed from the tables that the two amylase preparations in absence of either amino acid behave quite differently, the less active one, employed in connection with phenylalanine, being destroyed more rapidly with increasing temperature. Glycine added to a substrate hydrolyzed by this enzyme at 60°C. gave practically the same result as obtained with phenylalanine. Therefore, it appears that the lack of agreement in the results is due not to dissimilarity in the action of the two amino acids but rather to some difference in the amylases, probably connected with the deterioration which had already occurred in the less active preparation.

The most evident explanation of this marked temperature effect is that the amino acids preserve the enzyme in solution from the destructive influence of heat. In so far as the result of enzymic hydrolysis is concerned, increase in temperature exerts two opposite influences upon amylolytic action. It accelerates the velocity of conversion of starch into sugar and at the same time increases the rate of deterioration of the enzyme. The second reaction being retarded by the presence of one of the decomposition products, the first effect, that is, increase in the rate of hydrolysis of the starch, becomes more noticeable.

When hydrolysis continued for 60 minutes the amino acids produced a greater apparent activation at all temperatures until after coagulation of the enzyme had occurred than was observed for the shorter period digestions. Digestions carried out at 40°C. for periods of time from 20 minutes to 3 hours with and without glycine and tyrosine show the same increase in apparent activation with length of time of action of the amylase. This is what would be expected if the amino acid activates by protecting against deterioration, since the longer the enzyme is subjected to an injurious temperature, the greater the deterioration and consequently the more marked the activating effect in digestion mixtures in which the destruction is partially prevented.

The fact, demonstrated by the above data, that the presence of certain amino acids retards the deterioration of the enzyme constitutes an interesting addition to the evidence supporting the view that the enzyme itself is a substance of protein nature or which contains protein as an essential constituent.

SUMMARY

Addition of glycine, alanine, phenylalanine or tyrosine caused an undoubted increase in the rate of hydrolysis of starch by purified pancreatic amylase, commercial pancreatin, saliva or purified malt amylase. Less marked results were obtained with the less sensitive enzyme materials, malt extract, takadiastase and aspergillus amylase.

Each of the four amino acids here studied as well as aspartic acid and asparagine previously investigated, showed a similar favorable influence upon the enzymic hydrolysis of starch.

The addition of a mixture of two amino acids produced no greater effect than would result from the same concentration of one of them.

In these experiments the favorable effect of the added amino acid was not due to any influence upon hydrogen ion concentration nor to combination of the amino acid with the products of the enzyme reaction.

The addition of one of these amino acids is a very effective means of protecting the enzyme from the deleterious influence of cupric sulphate and may even serve to restore to full activity an enzyme which has been partially inactivated by copper.

The favorable influence of the amino acid is evidently due in part at least to a protection of the enzyme from deterioration in the aqueous solution in which it acts. This view is supported by the following facts: (1) Solutions of pancreatic amylase (containing optimum concentrations of chloride and phosphate) which have stood 1 hour at 40°C. show ⅓ greater activity when alanine has been added to the solution in advance. Amylase solutions to which the chloride and phosphate have not been added are protected by alanine at 22°C. (2) There is a striking increase in activation by glycine and phenylalanine with increased temperature until coagulation of the enzyme occurs. (3) At the same temperature, there is greater apparent activation when hydrolysis is allowed to proceed for 1 hour than when the action is stopped at the end of 30 minutes.

This explanation of the mode of action of amino acid does not preclude the possibility of a more direct influence upon the activity of the enzyme.

VITA

Florence Walker was born at Oriole, Md., October 15, 1887. She was prepared for college in the Blackstone School for Girls, Blackstone, Va. Entering Randolph-Macon Woman's College in 1905 she received the degree of Bachelor of Arts in 1909. Until 1917 she was Instructor in Chemistry in Randolph-Macon. During the years 1917-18 and 1921-22 and Summer Sessions 1916 and 1917 she pursued the study of chemistry in Columbia University, receiving the degree of Master of Arts in 1918. From 1918 to 1921 she was Carnegie Research Assistant to Professor H. C. Sherman, during which period she was co-author with Professor Sherman of several papers on amylases. For the past year she has held a University Fellowship in Chemistry.